AMENDMENTS TO THE CLAIMS

1. **(Currently Amended)** A computer-implemented method for counting nucleic acid probe signals in a region of interest in a biological specimen, the method comprising:

obtaining, by use of a confocal microscope, a plurality of successive two-dimensional image slices of the region of interest taken at different depths along a z-axis via the confocal microscope, wherein the successive two-dimensional image slices represent respective optical sections of the region of interest at different depths of the biological specimen;

with the plurality of successive two-dimensional image slices of the region of interest **taken at different depths along a z-axis via the confocal microscope**, distinguishing spatially overlapping nucleic acid probe signals in the biological specimen**, wherein distinguishing comprises transforming the successive two-dimensional image slices into transformed digital representations of the two-dimensional image slices and combining, via the transformed digital representations of the two-dimensional image slices, contiguous signal segments in successive optical sections into a single spot representing a single probe**;

in a computer system, **determining a number of test spots, wherein determining the number of test spots comprises** automatically counting ~~a~~ **the** number of test **spots represented in the successive two-dimensional image slices for** signals from a test probe;

in the computer system, **determining a number of reference spots, wherein determining the number of test spots comprises** automatically counting ~~a~~ **the** number of reference **spots represented in the successive two-dimensional image slices for** signals from a reference probe; and

in the computer system, determining a ratio of the **number of** automatically-counted test ~~signals~~ **spots** from the test probe to the **number of** automatically-counted reference ~~signals~~ **spots** from the reference probe, wherein the region of interest comprises multiple cells**, and the ratio indicates a ratio of the number of test probes to the number of reference probes**;

wherein the automatically-counted test **spots for** signals from the test probe comprise at least two distinguished spatially overlapping nucleic acid probe ~~signals~~ **spots** in the biological specimen.

2. **(Currently Amended)** The method of claim 1, wherein the reference probe is a polynucleotide that hybridizes to a centromere, and the number of reference **spots represented in the successive two-dimensional image slices for** signals from the reference probe approximates a nucleus count in the biological specimen.

3. (Original) The method of claim 1, wherein the reference probe recognizes a target on a same chromosome as the test probe.

4. (Original) The method of claim 1, wherein the test probe is a polynucleotide that hybridizes to a target sequence in a gene, and the reference probe is a polynucleotide that hybridizes to a reference sequence.

5. (Previously presented) The method of claim 4, wherein the reference probe recognizes a centromere of the same chromosome on which the gene of interest is contained.

6-8. (Canceled)

9. **(Currently Amended)** The method of claim 1, wherein the successive two-dimensional image slices are transformed into digital representations in which contiguous signal segments in successive optical sections are combined into a single ~~signal~~ **spot** in a particular optical section in which a strongest signal segment is located.

10. **(Currently Amended)** The method of claim 1, wherein different successive two-dimensional image slices are obtained for the test probe signals and the reference probe signals, and a quantity of test **probe spots for test** probe signals and reference **probe spots for reference** probe signals are determined.

11. **(Currently Amended)** The method of claim 1, wherein successive two-dimensional

image slices are obtained which show distinguishable test **probe spots for test** probe signals and reference **probe spots for reference** probe signals, and a quantity of the test **probe spots for test** probe signals and reference **probe spots for reference** probe signals are determined.

12. (Canceled)

13. **(Currently Amended)** The method of claim 1, wherein the ratio of ~~**signals**~~ **spots** is determined without reference to boundaries of a cell nucleus.

14. **(Currently Amended)** The method of claim 1, wherein the ratio of ~~**signals**~~ **spots** is determined without reference to the boundaries of a cell.

15. (Withdrawn-currently amended) The method of claim 1, wherein the probe ~~**signals**~~ **spots** are visible **spots from** signals from probes used with in situ hybridization of a biological sample, the method further comprising:

obtaining a plurality of images at different levels of the biological sample; and

constructing a three-dimensional image indicating discrete ~~**signals**~~ **spots** at different levels of the three-dimensional image;

wherein automatically counting comprises counting computer-identified discrete ~~**signals**~~ **spots** out of the discrete ~~**signals**~~ **spots** at different levels of the three-dimensional image.

16. (Withdrawn-currently amended) The method of claim 15, wherein the three-dimensional image is constructed by determining a location of a signal segment in the different levels of the biological sample, combining overlapping signal segments in contiguous levels into a single spot ~~**signal**~~, and separating signal segments in non-contiguous levels into different spots.

17. (Withdrawn) The method of claim 16, wherein the location of signal segments is determined by the presence of an increase in brightness intensity that indicates an increase of signal as compared to a background signal.

18. (Withdrawn) The method of claim 17, wherein the probes display fluorescent signals, and the increase in brightness intensity is associated with an increase in fluorescence compared to the background signal.

19. (Withdrawn-currently amended) The method of claim 15, wherein the ~~**signals**~~ **spots** comprise test **spots for test** signals from a test probe and reference **spots for reference** signals from a reference probe.

20. (Withdrawn) The method of claim 19, wherein the test probe recognizes a gene of interest, and the reference probe recognizes a chromosomal locus having an expected quantity in the biological specimen.

21. (Withdrawn-currently amended) The method of claim 20, further comprising determining a ratio between the test ~~**signals**~~ **spots** and the reference ~~**signals**~~ **spots**.

22. (Withdrawn-currently amended) The method of claim 21, further comprising determining:

(a) whether there is an increase in an expected ratio between the test ~~**signal**~~ **spots** and the reference ~~**signal**~~ **spots**, indicating an amplification of the gene of interest; or

(b) whether there is a decrease in the expected ratio between the test ~~**signal**~~ **spots** and the reference ~~**signal**~~ **spots**, indicating relative loss of the gene of interest.

23. (Withdrawn) The method of claim 19, wherein the test probe is selected from the group consisting of probes that recognize genes implicated or suspected in the development or progression of a tumor.

24. (Withdrawn) The method of claim 15, wherein the biological sample is in a microarray.

25. (Withdrawn) The method of claim 24, wherein the microarray comprises a tissue microarray.

26. (Withdrawn) The method of claim 25, wherein the tissue microarray comprises tissue samples of a same tissue type taken from a plurality of donor specimens.

27. (Withdrawn) The method of claim 15, wherein the plurality of images consists of between eight and thirty two images at different levels of the biological sample.

28. (Withdrawn-currently amended) The method of claim 15, further comprising:
avoiding counting discrete ~~signals~~ **spots** having intensities exceeding a threshold intensity.

29. (Withdrawn-currently amended) The method of claim 15, further comprising:
avoiding counting discrete ~~signals~~ **spots** having a combined intensity and area exceeding a threshold value.

30. (Withdrawn-currently amended) The method of claim 15, further comprising:
avoiding counting discrete ~~signals~~ **spots** related to autofluorescent material.

31. (Withdrawn) The method of claim 15, further comprising:
depicting a two-dimensional image representing the three-dimensional image for consideration by a user.

32. (Withdrawn-currently amended) The method of claim 31, further comprising:
emphasizing discrete ~~signals~~ **spots** related to autofluorescent material in the two-dimensional image.

33. (Withdrawn-currently amended) The method of claim 15, further comprising:
identifying a set of one or more discrete ~~signals~~ **spots** as a cluster; and
counting the cluster as a number of discrete ~~signals~~ **spots** greater than the number of discrete ~~signals~~ **spots** in the set.

34. (Withdrawn-currently amended) The method of claim 33, wherein the cluster is counted as a number of discrete ~~signals~~ spots indicated by applying a mapping to the number of discrete ~~signals~~ spots in the set.

35. (Withdrawn-currently amended) The method of claim 33, wherein the cluster is counted as a number of discrete ~~signals~~ spots indicated by a function calibrated via manual counting of spots in a plurality of images.

36. (Withdrawn-currently amended) The method of claim 33 wherein the cluster is counted as a number of discrete ~~signals~~ spots indicated by a gain factor applied to the number of discrete ~~signals~~ spots in the set.

37. (Withdrawn-currently amended) The method of claim 15, wherein the plurality of images are a set of images taken during a first analysis of a first color channel, and a second set of images are taken of the biological sample for a second color channel, the method further comprising:

avoiding counting discrete ~~signals~~ spots appearing at a same location in the set of images for the first color channel and the set of images in the second color channel.

38. (Withdrawn-currently amended) The method of claim 15, wherein the plurality of images are a set of images taken for a test probe, and a second set of images are taken of the biological sample for a reference probe, the method further comprising:

avoiding counting discrete ~~signals~~ spots appearing at a same location in the set of images for the test probe and the set of images for the reference probe.

39. (Withdrawn-currently amended) The method of claim 15, further comprising:

receiving a directive from a user indicating counting is to be avoided for a specified portion of the biological sample; and

responsive to the directive, avoiding counting discrete ~~signals~~ spots for the specified portion of the biological sample.

40. (Withdrawn-currently amended) The method of claim 15, further comprising:

receiving a directive from a user indicating counting is to be performed separately for a specified portion of the biological sample; and

responsive to the directive, separately counting discrete ~~**signals**~~ **<u>spots</u>** for the specified portion of the biological sample.

41-63. (canceled)

64. **(Currently Amended)** An automated system for counting nucleic acid probe signals in a region of interest in a biological specimen, the system comprising:

means for obtaining, by use of a confocal microscope, a plurality of successive two-dimensional image slices of the region of interest taken at different depths along a z-axis via the confocal microscope, wherein the successive two-dimensional image slices represent respective optical sections of the region of interest at different depths of the biological specimen;

~~**means for counting a number of test signals from a test probe;**~~

~~**means for counting a number of reference signals from a reference probe;**~~

means for distinguishing spatially overlapping nucleic acid probe signals in the biological specimen via the plurality of successive two-dimensional image slices of the region of interest **<u>taken at different depths along a z-axis via the confocal microscope, wherein distinguishing comprises transforming the successive two-dimensional image slices into transformed digital representations of the two-dimensional image slices and combining, via the transformed digital representations of the two-dimensional image slices, contiguous signal segments in successive optical sections into a single spot representing a single probe;</u>**

<u>means for counting a number of test spots represented in the successive two-dimensional image slices for signals from a test probe, whereby a number of test spots is determined;</u>

<u>means for counting a number of reference signals from a reference probe, whereby a number of reference spots is determined</u>; and

means for determining a ratio ~~**of**~~ **<u>for</u>** the **<u>number of</u>** counted test ~~**signals**~~ **<u>spots</u>** from the test probe ~~**to**~~ **<u>and</u>** the counted reference ~~**signals**~~ **<u>spots</u>** from the reference probe, wherein the region of interest comprises multiple cells**<u>, and the ratio indicates a ratio for the number of test probes and</u>**

the number of reference probes;

wherein the counted test **spots for** signals from the test probe comprise at least two distinguished spatially overlapping nucleic acid probe ~~signals~~ **spots** in the biological specimen.

65. (Canceled)

66. (Canceled)

67. (Previously Presented) One or more computer-readable media comprising computer-executable instructions for performing the method of claim 9.

68. **(Currently Amended)** One or more computer-readable media having computer-executable instructions for performing a method for counting nucleic acid probe signals in a region of interest in a biological specimen, the method comprising:

obtaining via confocal microscopy a plurality of successive two-dimensional image slices of the region of interest taken at different depths along a z-axis via the confocal microscopy, wherein the successive two-dimensional image slices represent respective optical sections of the region of interest at different depths of the biological specimen;

with the plurality of successive two-dimensional image slices of the region of interest **taken at different depths along a z-axis via the confocal microscope**, distinguishing spatially overlapping nucleic acid probe signals in the biological specimen**, wherein distinguishing comprises transforming the successive two-dimensional image slices into transformed digital representations of the two-dimensional image slices and combining, via the transformed digital representations of the two-dimensional image slices, contiguous signal segments in successive optical sections into a single spot representing a single probe**;

determining a number of test spots, wherein determining the number of test spots comprises automatically counting ~~a~~ **the** number of test **spots represented in the successive two-dimensional image slices for** signals from a test probe;

determining a number of reference spots, wherein determining the number of test spots comprises automatically counting ~~a~~ **the** number of reference **spots represented in the successive**

two-dimensional image slices for signals from a reference probe; and

determining a ratio of the **number of** automatically-counted test ~~signals~~ **spots** from the test probe to the **number of** automatically-counted reference ~~signals~~ **spots** from the reference probe, wherein the region of interest comprises multiple cells**, and the ratio indicates a ratio of the number of test probes to the number of reference probes**;

wherein the automatically-counted test **spots for** signals from the test probe comprise at least two distinguished spatially overlapping nucleic acid probe ~~signals~~ **spots** in the biological specimen.

69. **(New)** The method of claim 1 further comprising:

creating a stack of binary images for the two-dimensional image slices; and

grouping binary spot markers occurring as vertical neighbors in the stack into a single spot representing a single probe.

70. **(New)** The method of claim 1, wherein the combining comprises:

assigning the single spot to a particular optical section or sections.